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⑥④ **Fat composition.**

⑥⑦ Substitute milk fat especially as a replacement fat in infant formulations comprises 2-saturated glycerides especially 2-palmitic acid glycerides, in which the 1, 3 positions are randomly occupied substantially by different shorter chain and/or unsaturated fatty acids. These glycerides are prepared by selective rearrangement of glycerides using 1, 3-regio-specific lipase enzymes as rearrangement catalysts, preferably in acidolysis rearrangement using unsaturated acid or alkyl ester thereof.

**EP 0 209 327 A2**

FAT COMPOSITIONS

This invention relates to substitute milk fat  
5 compositions, for replacing at least part of the milk fat  
necessary to feed young mammals and especially infants.  
The invention also relates to their preparation by  
rearrangement of fats using lipase enzymes as  
rearrangement catalysts, and to milk substitutes  
10 containing such fats.

Milk replacement fats should match the performance of  
milk fat as closely as possible in order to reproduce its  
physical and dietary characteristics. Human milk fat  
15 consists of a variety of triglycerides of both saturated  
and unsaturated fatty acids, the former being chiefly  
palmitic acid with a smaller amount of stearic and  
myristic acids and a small but significant amount of  
shorter chain acids. The unsaturated acids consist  
20 substantially of oleic with smaller quantities of linoleic  
and palmitoleic acid and a little linolenic acid.

Infant formulations are based on fat, carbohydrate  
and protein, together with added vitamins, essential

minerals and other minor components. The proportions of these and the major components has been adjusted from time to time in an effort to develop a formula more nearly approximating to mothers' milk, and where special formulae were needed for premature infants or those with metabolic difficulties. For example, skim milk may be replaced by soy isolate as the protein source for infants with cows' milk sensitivity and lactose as the carbohydrate may be replaced by other sugars and starches. For the fat component, both animal and vegetable fats have been used.

Fat compositions containing similar amounts of the principal fatty acids of milk fat may be derived from oils and fats of vegetable origin. With the notable exception of so-called lauric fats such as coconut and palm kernel oils, most vegetable oils and fats consist substantially of  $C_{16}$  and  $C_{18}$  fatty acids, although shorter-chain fatty acids characteristic of milk fat are notably absent. The non-lauric vegetable fats and oils also tend to be more highly unsaturated than milk fat - at any rate that of land-based mammals. Nevertheless, fats may be derived with generally similar fatty acid composition to milk fat.

A significant difference nevertheless remains which is believed to have important dietary consequences; most glycerides of vegetable origin are overwhelmingly unsaturated in the 2- position, chiefly oleic and linoleic acids. In contrast, substantial amounts of palmitic acid occupy the 2- position of glycerides in human milk fat, more than half the total fatty acid there being palmitic acid, and the preponderance of total palmitic acid in the fat being located in this position, the major glyceride of human milk fat being 1, 3 di-oleoyl-2-palmitoyl glyceride. Randomisation of vegetable-based fats containing palmitic acid residues leave insufficient in the 2-position of the product to provide a good match, without placing excessive amounts of palmitic acid in the other positions, thereby

including unwanted glycerides in the composition. Fractional crystallisation to remove glycerides containing palmitic acid in the 1, 3-positions is tedious, expensive and usually unsuccessful.

5

The distribution of fatty acids and the triglycerides of some milk fats of nutritional importance was studied by Freeman, Jack and Smith (J. Dairy Sci., 1965, p.853), who reported that in human milk fat a greater proportion of  
10 palmitic acid appears in the 2-position and of stearic acid and oleic acid in the 1, 3 positions than in the milk fat or ruminants. The greater absorption of palmitic acid in the 2-position of triglycerides by infants was reported by Filer, Mattson and Fomen (J. Nutrition, 99, pp  
15 293-298), who suggested that the relatively poor absorption of butter fat by infants compared with human milk fat is attributable to its substantially uniform distribution of palmitic acid between the glyceride positions of the fat. Experiments shows that about 95%  
20 unrandomised lard could be absorbed by infants compared with about 70% for randomised lard, in which less palmitic acid appears in the 2-position. The conclusion that fatty acid is better absorbed in the 2-position is believed to hold for all fatty acids, but palmitic acid is of  
25 particularly importance for infants and stearic acid to a lesser extent.

The present invention provides a novel fat composition suitable for replacing at least part of the  
30 fat in infant food formulations comprising a mixture of glycerides wherein different fatty acid residues in the 1- and 3- positions are randomly distributed between these positions and include unsaturated residues, wherein at least half of those in the 2- position are C<sub>16</sub> and/or C<sub>18</sub>  
35 saturated, preferably consisting substantially of palmitic acid, particularly 60-90% by weight of the total

2-position fatty acids. All the fatty acids, or virtually all, should be unbranched and even-numbered.

5 The 1-, 3- positions of the novel compositions  
according to the invention include unsaturated fatty  
acids. These should preferably consist largely of oleic  
acid with linoleic acid and palmitoleic and less than 1%  
others. Preferably also the compositions include at least  
10 as much saturated fatty acid in the 2-position as in the  
1- and 3- positions combined, more preferably up to twice  
as much. Preferably also the 1, 3 positions include both  
unsaturated  $C_{18}$  and saturated  $C_4$  to  $C_{14}$  fatty acids. The  
proportion and variety of these fatty acids may be  
determined in accordance with dietary and physical  
15 requirements of the composition required. Milk replacement  
fats should be capable of emulsification at blood heat in  
liquid feed and should therefore preferably be melted at  
this temperature. The melting point of fats is determined  
by their fatty acid composition which may be selected  
20 accordingly.

Other fats, particularly lauric fats, may be included  
in the compositions of the invention to provide blends  
matching the composition of milk fat or its melting  
25 characteristics, and also vegetable oils for example  
sunflower oil and soya bean oil, having a high content of  
polyunsaturated fatty acid glycerides, to improve the  
dietary benefit of the compositions of the invention.

30 Novel compositions according to the invention may be  
obtained by rearrangement of fatty mixtures comprising  
glycerides consisting substantially of more saturated 2-  
palmityl glycerides, under the influence as rearrangement  
catalyst of an enzyme lipase which is regiospecific in  
35 activity in the 1 and 3 positions only of glycerides.  
Processes of this kind are described in our British patent

specification 1577933. Under the influence of the catalyst, unsaturated fatty acid residues may be introduced into the 1- and 3-positions of the 2- palmityl glycerides by exchange with other glycerides or more preferably by means of acidolysis reagents in the fatty mixture, particularly unsaturated free fatty acids preferably oleic acid or their alkyl esters. Shorter chain saturated acids may alternatively be introduced. The 2- palmityl glycerides modified in this way may then be separated from the product. A mixture of acidolysis reagents may be used to provide a mixture of glycerides in the product conforming substantially to that of milk fat. The reaction is preferably carried out at a temperature from 10 to 90°C and maybe perfumed to batchwise or incontinuous fashion, with or without a water-immiscible organic solvent.

A suitable source of 2- palmityl glyceride for use in this process may be obtained as a top fraction of palm oil, which contains up to 12% trisaturated acid glycerides including tripalmitin. Generally a top fraction contains about 4 parts tripalmitin and 1 part symmetrical disaturated triglycerides.

The novel compositions of the invention comprise a symmetrical 2-saturated  $C_{16}/C_{18}$  glyceride mixture, the 1- and 3-positions containing statistically the same fatty acid residue composition comprising more than one fatty acid.

The present invention also provides infant food compositions comprising fat, protein and carbohydrate components in the approximate relative weight proportions 2.5:1:5, wherein at least part of the fat normally used in such formulations is replaced by an enzyme-rearranged fat in accordance with the present invention. Dry

formulations containing this component mixture, together with additional components customary in such formulations, should be dispersed for use in sufficient water to produce  
5 an emulsion of approximately  $3\frac{1}{2}$  grams of fat per 100 mls. of dispersion.

EXAMPLE 1

10 Calculations appear in the following Table I for the analysis of three glyceride compositions that may be provided by rearrangement of a mixture of a top fraction of palm oil with either oleic acid in the proportion 3:1 (Sample 2) or an alternative fatty acid composition (FAM)  
15 used in proportions of 2:1 and 3:1 by weight of the feedstock (Samples 1 and 3). The mixture was rearranged at 40°C in a solution in hexane, using Mucor miehei enzyme as rearrangement catalyst. The catalyst was supported on Celite and activated by slurring with water before use.  
20 The method used was in accordance with our British patent specification No. 1577933. Free fatty acid was removed from the reactio product by steam stripping.

The fatty acid composition (FAM) consisted of:-  
25 Palmitoleic 6; stearic 12; oleic 69; linoleic 10 and others 2 and the palm top fraction was 80% P<sub>3</sub> and 20% POP (P = palmitic; O = oleic).

TABLE I

5	GLYCERIDE SUBSTITUENT(S)						SAMPLE NO.
	C <sub>16:0</sub>	C <sub>16:1</sub>	C <sub>18:0</sub>	C <sub>18:1</sub>	C <sub>18:2</sub>		
	1,3	27	5	9	52	7	
10	2:1						
	2	80	-	-	20	-	1
	TOTAL						
	1,2,3	44.5	3.5	6	41.5	4.5	
15							
	1,3	20/			80/		
		20	-/5	-/10	57	-/8	
	3:1 2	80/			20/		
20		80	/-	/-	20	/-	2/3
	TOTAL	40/			60/		
	1,2,3	40	/3.5	/6.5	44.5	/5.5	
25							

Blends were made of samples (1), (2) and (3) with sunflower (SF) palm kernel (PK) and soya bean oils (SB) as shown in Table 2.



TABLE 2

	<u>Blend</u>	<u>Product</u>	<u>SF</u>	<u>PK</u>	<u>SB</u>
5	1	(1) 70	10	20	
	2	(1) 50	10	10	30
	3	(2) 70	20	10	
	4	(3) 70	10	20	

10        The blends are compared with the composition of human milk in the following Table 3.

TABLE 3

5		12:0	14:0	16:0	16:1	18:0	18:1	18:2
	GLYCERIDE SUBSTITUENTS 1,2,3							
	MILK FAT	2.3	5.0	25.7	5.1	7.1	38.5	11.7
	10 BLENDS	1	9.5	3.0	33.0	2.0	5.5	35.0
		2	5.0	2.0	26.0	1.5	5.0	33.5
15		3	5.0	1.5	30.0	N.D.	1.0	47.5
		4	10.0	3.0	30.5	2.5	5.0	36.5

TABLE 3 (CONT.)

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TABLE 3 (CONT)

GLYCERIDE								
SUBSTITUENT 2								
<hr/>								
MILK FAT		1.6	6.5	57.5	4.6	3.6	13.8	7.9
10	BLENDS 1	9.5	3	57.0	3.5	8.0	22.0	11.5
	2	5.5	1.5	41.0	N.D.	N.D.	23.5	26.5
	3	5.0	1.5	56.5	N.D.	N.D.	21.0	16.0
	4	10.0	3.0	57.0	N.D.	N.D.	22.0	8.0
<hr/>								
15								

TABLE 3 (CONT)

5	GLYCERIDE									
	SUBSTITUENTS 1, 3									
	MILK FAT	2.6	4.3	10.3	5.3	8.8	50.3	13.5		
10	BLENDS 1	9.5	3.0	21.0	3.0	7.5	41.5	11.5		
	2	5.0	2.0	19.0	2.5	7.0	39.0	21.5		
	3	5.0	1.5	17.0	N.D.	1.5	61.0	14.0		
	4	10.0	3.0	17.5	3.5	7.5	43.5	12.0		

N.D. = NONE DETECTED

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From Table 3 it will be apparent that good similarity is obtained in fatty acid content and distribution with milk fat, particularly by blends 3 and 4. A high 2-palmitic acid distribution is obtained in all the blends, matching that of milk fat, without unduly increasing the palmitic acid in the other glyceride positions. Lauric acid is consistently higher all round, providing a dietary advantage for infants, while myristic acid is marginally lower. Of the longer-chain acids, palmitoleic and stearic are present in similar amounts with a similar distribution except in the 2-position, while oleic acid is significantly higher in the 2-position in the blends, which also showed generally high linoleic acid in the 2-position but not overall.

In all the above Tables percentages are by weight. Milk fat compositions are based on data reported in mole per cent, assuming an average molecular weight for unspecified fatty acids of  $C_{15:0}$ . Summations less than 100% are accounted for by the presence of other acids.

#### EXAMPLE 2

A mixture of 100 grammes of an upper-melting fraction of palm oil consisting substantially of 1 part tripalmitin and 4 parts 1, 3-dipalmitoyl-2-oleoyl glyceride with 200 grammes of oleic acid was dissolved in 400 grammes of hexane. 25 grammes of rearrangement catalyst was added and the mixture agitated at 55°C for 24 hours. The catalyst comprised Mucor Miehei lipase enzyme deposited on Celite in the manner described in our British patent specification no. 1577933 Case A.171. The catalyst was pre-activated by the addition of 10% water by weight of the catalyst.

After separating the catalyst by filtration and distilling off the solvent, the residue remaining was distilled in a molecular still at 235°C and  $6 \times 10^{-3}$  mm. Hg. to remove free fatty acid as a distillate amounting to 80% by weight of the total residue. The acid distillate and triglyceride fraction remaining were analysed for free fatty acid and partial glyceride, and the triglyceride composition was further analysed for individual triglycerides. Particulars of these analyses are given in Table 4.

TABLE 4

<u>Fraction</u>	SSS	SOS	SSO	SlnS	S00	OS0	Others
Triglyceride	9.5	6.5	23.9	5.1	15.7	21.5	18.7

Blends were made of the triglyceride fraction with additional fats to emulate the composition of human milk fat, blend A containing 10 and 20% respectively of sunflower oil and palm kernel oil and blend B containing 10% each of sunflower and palm kernel oil, with 30% of soyabean oil.

In Table 5 the fatty acid content of the triglyceride fraction and the blends made from it is compared with human milk, showing in particular that a good match is made by the blends with the principal acids in human milk fat. In Table 6 the corresponding data appears for fatty acids in the 2-position and shows that the triglyceride fraction contains substantially the same amount of palmitic acid in the 2-position as human milk fat and that, while a drop is suffered in the blends, a substantial amount remains in this position.

Corresponding solid content values measured by pulse NMR methods were obtained and appear in Table 7 where they are compared with butter oil which is used extensively in the preparation of baby milk formulations.

TABLE 5

5	<u>FATTY ACID</u>	12:0	14:0	16:0	16:1	18:0	18:1	18:2	OTHERS
	HUMAN MILK	2.3	5.0	25.7	5.1	7.1	38.5	11.7	4.6
	BLEND A	12.9	4.7	30.2	2.0	3.2	34.5	10.6	1.9
	BLEND B	6.3	2.6	24.4	1.5	3.4	33.6	24.4	3.7
	ENZYME PRODUCT	0.6	1.8	41.0	3.1	3.0	43.6	4.4	2.5

TABLE 6

15	<u>2 POSITION</u>	12:0	14:0	16:0	16:1	18:0	18:1	18:2	OTHERS
	<u>FATTY ACID</u>	1.6	6.5	57.5	4.6	3.6	13.8	7.9	4.5
	HUMAN MILK	9.0	4.2	37.0	1.4	3.5	30.3	13.3	1.3
	BLEND A	4.5	2.1	25.5	1.4	3.0	27.6	30.0	5.6
	BLEND B	-	1.4	55.2	2.3	4.0	32.9	4.2	-
	ENZYME PRODUCT	-	-	-	-	-	-	-	-



5

TABLE 7

TEMP °C	0	5	10	15	20	25	30	35	40
BLEND A	54	52	45	32	21	11	7	4	3
BLEND B	37	35	28	18	12	6	4	2	1
10 ENZYME PRODC	61	59	54	41	30	19	13	8	4
BUTTER OIL	53	49	42	29	15	9	5	1	0

CLAIMS

1. Fat composition comprising a mixture of glycerides wherein at least half the fatty acid residues in the  
5 2-position are saturated and 1, 3-position fatty acid residues are randomly distributed between these positions and include other fatty acid residues.
2. Fat composition according to claim 1 wherein the  
10 saturated fatty acid in the 2-position consists substantially of palmitic acid.
3. Fat composition according to claim 1 or 2 wherein the  
15 1, 3-position fatty acids include not more than 20% palmitic acid.
4. Fat composition according to any preceding claim and containing at least as much saturated acid residues in the  
20 2-position as in the 1, 3-positions combined.
5. Fat composition according to claim 4 containing in the 2-position up to twice the amount of saturated fatty acid in the 1, 3-positions combined.
- 25 6. Symmetrical 2-palmityl unsaturated fats containing at least two fatty acid residues in the 1, 3-positions.
7. Fat composition according to any of the preceding claims wherein the 1, 3-position fatty acids include both  
30 unsaturated  $C_{18}$  and saturated  $C_4$  to  $C_{12}$  fatty acids.
8. Substitute milk fat composition comprising a fat composition as claimed in any of the preceding claims in a blend including a lauric fat and a vegetable oil at least  
35 40% of the fatty acids of which contain two or more double bonds.

9. Composition as claimed in claim 8 comprising 10 to 20% lauric fat.
10. Composition as claimed in claim 8 or 9 comprising 10 to 30% of said vegetable oil.
11. Composition as claimed in claim 8-10 wherein the lauric fat is palm kernel oil and/or the vegetable oil is sunflower oil or soya bean oil.
12. Composition according to claims 8 to 11 wherein the Solids Content Index is within the ranges 35 to 55 at 0°C, 25 to 50 at 10°C, 10 to 25 at 20°C and not more than 10 at 30°C.
13. Infant food composition comprising approximately 1 part protein, 5 parts carbohydrate and 2½ parts fat, wherein at least part of the fat comprises a composition as claimed in any of the preceding claims 1 to 12.
14. Process for the preparation of fats according to claim 1 comprising rearranging a mixture of glycerides including substantial amounts of 2-palmityl glycerides, in the presence of an unsaturated fatty acid and an activated catalyst which is 1, 3-regiospecific.
15. Process according to claim 14 wherein the glyceride mixture comprises a palm fat.
16. Process according to claim 14 or 15 wherein the unsaturated acid comprises oleic acid.
17. Process according to any of the preceding claims 13 to 16 wherein the rearranged fat is fractionated to recover an unsaturated, symmetrical mixture of 2-palmityl glycerides.

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18. Process according to claim 8, substantially as herein  
before described with reference to the accompanying  
examples.

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